ELSEVIER

BioSystems 54 (2000) 107–130

BioSystems

www.elsevier.com/locate/biosystems

Free energy and information contents of *Conformons* in proteins and DNA

Sungchul Ji *

Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ 08855, USA

Received 11 September 1998; received in revised form 8 September 1999; accepted 4 October 1999

Abstract

Sequence-specific conformational strains (SSCS) of biopolymers that carry free energy and genetic information have been called *conformons*, a term coined independently by two groups over two and a half decades ago [Green, D.E., Ji, S., 1972. The electromechanochemical model of mitochondrial structure and function. In: Schultz, J., Cameron, B.F. (Eds.), Molecular Basis of Electron Transport. Academic Press, New York, pp. 1–44; Volkenstein, M.V., 1972. The Conformon. J. Theor. Biol. 34, 193–195]. Conformons provide the molecular mechanisms necessary and sufficient to account for all biological processes in the living cell on the molecular level in principle — including the *origin of life*, *enzymic catalysis*, *control of gene expression*, *oxidative phosphorylation*, *active transport*, and *muscle contraction*. A clear example of SSCS is provided by SIDD (strain-induced duplex destabilization) in DNA recently reported by Benham [Benham, C.J., 1996a. Duplex destabilization in superhelical DNA is predicted to occur at specific transcriptional regulatory regions. J. Mol. Biol. 255, 425–434; Benham, C.J., 1996b. Computation of DNA structural variability — a new predictor of DNA regulatory regions. CABIOS 12(5), 375–381]. Experimental as well as theoretical evidence indicates that conformons in proteins carry 8–16 kcal/mol of free energy and 40–200 bits of information, while those in DNA contain 500–2500 kcal/mol of free energy and 200–600 bits of information. The similarities and differences between *conformons* and *solitons* have been analyzed on the basis of the *generalized Franck-Condon principle* [Ji, S., 1974a. A general theory of ATP synthesis and utilization. Ann. N.Y. Acad. Sci. 227, 211–226; Ji, S., 1974b. Energy and negentropy in enzymic catalysis. Ann. N.Y. Acad. Sci. 227, 419–437]. To illustrate a practical application, the conformon theory was applied to the molecular-clamp model of DNA gyrase proposed by Berger and Wang [Berger, J.M., Wang, J.C., 1996. Recent developments in DNA topoisomerases II structure and mechanism. Curr. Opin. Struct. Biol. 6(1), 84–90], leading to the proposal of an eight-step molecular mechanism for the action of the enzyme. Finally, a set of experimentally testable predictions has been formulated on the basis of the *conformon theory*.

Keywords: Conformational strains; Free energy; Information; Frank-Condon principle; Stress-induced duplex destabilization; DNA topoisomerases

1. Introduction

In the early 1970s, four groups independently introduced the concept of *conformons* or *confor-*

* Tel.: +1-732-445-4701; fax: +1-732-445-0119.
E-mail address: sji@eohsi.rutgers.edu (S. Ji)

0303-2647/00/$ - see front matter
PII: S0303-2647(99)00073-8

mon-like entities into bioenergetics, the study of energy transduction in living systems. The term conformon was formed from 'conform-' indicating conformation of a biopolymer and '-on' meaning a particle or unit. Conformons refer to mobile or transient *conformational strains* or *deformations* of biopolymers involved in enzymic catalysis, charge transport, and energy coupling such as active transport and muscle contraction. Volkenstein (1972) defined the conformon as conformational deformations of proteins caused by the displacement of an electron or of the electronic density in a macromolecule and likened it to the polaron in solid state physics. He called our attention to the fundamental significance of the coupling between fast electronic transitions (i.e. configurational or covalent changes) and slow nuclear rearrangements (i.e. conformational changes) attending enzymic catalysis (Volkenstein, 1972, 1981, 1986; Volkenstein et al., 1972; Shaitan and Rubin, 1982; see also Section 7 below). Green and Ji (1972), in contrast, characterized the conformon 'as the free energy associated with a localized conformational strain in biological macromolecule' and employed this concept to account for the molecular mechanisms underlying free energy storage in proteins during oxidative phosphorylation in mitochondria (Green and Ji, 1972; Ji, 1974a, 1976, 1977, 1979). The conformon mechanism was subsequently applied to enzymic catalysis (Ji, 1974b, 1979, 1991), active transport (Ji, 1974a, 1979), muscle contraction (Ji, 1974a), transcription and translation (Ji, 1990), and the origin of life (Ji, 1991). The term, *conformon*, was adopted by Kemeny and Goklany (1973, 1974) in their theoretical analysis of the semiconductive properties and compensation behaviors of biopolymers. Additionally, these authors developed the first quantum mechanical representation of the conformon concept (see also Zgierski, 1975). Finally, Davydov (1973, 1987) proposed the excitation of soliton-like vibrational motions of peptide groups in proteins (with the attendant conformational changes) as a possible mechanism for coupling ATP hydrolysis to the mechanical motions of myosin along the thin filament. Solitons refer to *solitary waves* formed in condensed media (liquids and solids) that can propagate and transport energy over long distances without dissipation (Scott et al., 1973; Rebbi, 1979; Lomdahl et al., 1984; Ji, 1985a; Scott, 1985; Shen, 1997). Solitons have been proposed as molecular mechanisms underlying not only muscle contraction (Davydov, 1973; Zgierski, 1975) but also energy and charge transport (Davydov, 1987; Ciblis and Cosic, 1997) through proteins, transient strand separations in DNA duplexes as manifested in rapid proton exchange between nucleotide bases and solvent protons (Englander et al., 1980), enzymic processes (Careri and Wyman, 1984), regulation of DNA transcription (Sobell et al., 1982; Polozov and Yakushevich, 1988), the origin of life (Careri and Wyman, 1985), and radiation-induced DNA strand breaks (Baverstock and Cundall, 1988). As will be discussed in Section 7, solitons can be viewed as constituting a subset of conformons; *i.e. all solitons are conformons*, *but not all conformons are solitons*.

The main objectives of this paper are fivefold: (1) To compare the free energy and information contents of conformons in proteins estimated previously (Ji and Finette, 1985; Ji, 1990) with the free energy and information contents of conformons in DNA calculated from the recent results of Benham (1990, 1992, 1993, 1996a,b); (2) to propose the notion that *conformons* are *quanta of biological actions* ultimately responsible for all molecular processes in living systems (Ji, 1985b,c) in analogy to *the quantum of action* of Max Planck that mediates all molecular interactions in physics and chemistry (Kauzmann, 1957; Pilar, 1968; Schiff, 1968; Bohm, 1979); (3) to delineate the similarities and differences between *conformons* and *solitons*; (4) to apply the conformon theory to the mechanism of action of DNA gyrase, and (5) to propose a set of experimentally testable predictions based on the conformon theory of biological information/energy transduction and transmission.

2. Information

It is impossible to define '*information*' in any simple way, without sacrificing some of its essential features. This is primarily because the concept

of information is *relational* and *context-dependent* (Küppers, 1996; Mahler, 1996). As a convenient mnemonic device, however, we may think of *information* as the ability to *control* or *influence work* or equivalently as the ability to *reduce uncertainty*, just as *energy* is often considered to be the ability to *do work*. To fix the notion of information more concretely, it may be helpful to compare the concept of information with the more familiar concept of energy as shown in Table 1.

Table 1
A qualitative comparison between energy and information

	Energy	Information
1. System	Thermodynamic systems Isolated Closed Open	Communication systems Sender (or message source) Channel Receiver (or destination)
2. Theory	Thermodynamics Statistical mechanics Quantum mechanics	Information theory Communication theory Complexity theory Biological theories Epistemological theories
3. Kinds	Thermal energies Gibbs free energy Helmhotz free energy Potential energy Kinetic energy, etc.	Syntactic information[a] Semantic information Pragmatic information
4. Law	First law of thermodynamics	'Principle of equivalence'[b]
5. Units	Calories Ergs Joules	Bits
6. Essential for work?	Yes	No
7. Essential for regulation?	Yes	Yes
8. Essential for reducing uncertainty?	Yes	Yes
9. Essential in physics?	Yes	No
10. Essential in biology?	Yes	Yes

[a] See Freund (1996) and Gernert (1996).
[b] See Küppers (1996).

Discussing energy without defining the thermodynamic system involved can lead to intractable confusions (e.g. the concept of the monotonic increase of entropy, i.e. the Second Law of Thermodynamics, applies only to isolated systems and not to closed or open systems). Similarly, any discourse on information will make sense only if discussants have a clear idea as to which aspect of a communication system is being implicated, since the information content of a message can differ significantly depending on whether it is viewed from the perspective of the sender or the receiver and also upon the nature of the codes agreed upon by the sender and the receiver.

The theories of energy, namely thermodynamics, statistical mechanics and quantum mechanics, have been well established in physics during the past two centuries. But it is only since the middle of this century that theories on information have been developing in earnest, including information theory and communication theory in engineering (Shannon, 1948; Wiener, 1948; Aczél and Daróczy, 1975; van der Lubbe, 1997), complexity theory in physics and mathematics (Zurek, 1990), biological theories on phylogeny and ontogeny (Wicken, 1987a,b; Ji, 1991; Yockey, 1992; Salthe, 1996), and epistemological theories in philosophy, cognitive sciences and linguistics (Kornwachs and Jacoby, 1996).

Just as there are numerous kinds of energies as indicated in Table 1, there are different kinds of information, called *syntactic*, *semantic*, and *pragmatic* informations (Freund, 1996; Gernert, 1996). Syntactic information is concerned with the structural and compositional relations among symbols, some of which can be precisely investigated using statistical and probabilistic methods. For example, one of the best known quantitative measures of information, known as *Shannon's entropy or information–theoretic entropy*, formulated more than a half century ago, belongs to this category (Shannon, 1948; Aczél and Daróczy, 1975; Pierce, 1980; van der Lubbe, 1997). It is the syntactic

aspect of information that will be the main focus of this paper (see below). The semantic aspect of information refers to the relation between symbols and their referents (also called objects or signifieds) and hence to the meaning of information. Finally, the pragmatic aspect of information has to do with the relation between symbols and the receiver or with the effects that the information transmitted through the symbols has on the receiver. It should be pointed out here that Shannon's information measure applies only to the amount and not the meaning nor the pragmatic aspects of information. In other words, Shannon's information measure is blind to the meaning or the pragmatic aspects of information, for which there are as yet no generally accepted quantitative measures available.

The first law of thermodynamics states that the energy of the Universe is conserved; i.e. energy can be neither created nor destroyed. In contrast, information can certainly be created (e.g. the emergence of living systems) or destroyed (e.g. extinction of species and senescence of organisms). According to Küppers (1996), because of the context-dependence of information, the generation of information always depends upon the prior existence of an equivalent amount of information. This notion is referred to as a '*principle of equivalence*.'

Some authors have claimed that information and energy can be interconverted (e.g. Stonier, 1990, 1996). A careful analysis of the paper by Stonier (1996) indicates that the idea of information–energy interconversion (as expressed in Eq. 4 in his paper) might have originated from the questionable assumption that '*organization*' (i.e. *Or* in Eq. (3) is identical with '*order*,' or the inverse of *disorder*, appearing in Boltzmann equation as modified by Schrödinger (1998) (see *Or* in Eq. (2)). Another crucial evidence that Stonier's conclusion may be in error comes from the fact that information and energy have different physical units (see row 5 in Table 1) and hence cannot be directly added to, nor subtracted from, each other.

Work is done when an object is displaced by a force applied to it. Energy expended on performing such a work is calculated by integrating the product of the differential distance traveled by the object and the magnitude of the force applied to it in the direction of its movement. So, the necessary and sufficient condition for performing work is the force and the displacement of an object to which the force is applied and hence no *information* (as defined by Eq. (2) below) is required for performing work (see row 6, Table 1). However, to perform *regulated* or *controlled* work, both *energy* and *information* would be required (see row 7, Table 1). In the example just cited, *information* would be required to specify the direction of the movement of the object and *energy* to move the object in any direction.

In a communication system, a signal being transmitted through the channel carries information from the sender to the receiver. Upon reaching the receiver, the information transmitted reduces the amount of the uncertainty in the knowledge of the receiver. Energy is required because without it no signal can be transmitted (Pierce, 1980); information is required because without it the receiver would experience no reduction in uncertainty (see row 8, Table 1).

Most of the fundamental developments in physics (thermodynamics, classical mechanics, quantum mechanics, statistical mechanics) in the last 100 years or so have occurred without any explicit implication of the concept of *information* (see row 9). In a stark contrast, the concept of information has played a major role in biology in the same period of time with the concept of energy playing a secondary role (see row 10). But there are theoretical reasons to believe that a full understanding of living processes (including enzymic catalysis, cell biology, physiology, development and evolution) may not be achieved without taking into account both the informational and energetic/material aspects of living phenomena on an equal footing (Wicken, 1987b; Ji, 1991, 1999a,b; Salthe, 1996). The study of both the energetic/material (i.e. thermodynamics, and statistical and quantum mechanics) and informational aspects (i.e. information theory) thought to be essential for biology has been variously called *biognergetics* (Ji, 1985c), *biocybernetics* (Ji, 1991), *infodynamics* (Salthe, 1996), or *molecular semiotics* (Ji, 1999b). It is predicted that *conformons*, which is the main focus of this paper, may play in the

emerging field of *molecular semiotics* (almost synonymous with biocybernetics, biognergetics and infodynamics) the role of *quantum of action* in quantum mechanics.

As already alluded to above, the term '*information*' in this paper will refer to the syntactic information only. As such, this information can be quantified using the 'information-theoretic entropy' equation formulated by Shannon in 1948 (Aczél and Daróczy, 1975; Pierce, 1980; van der Lubbe, 1997). Consider a nucleotide sequence, with the sample space X = (A, T, C, G), where the symbols in the parentheses indicate the usual four nucleotides, constituting the alphabet. If the probability of the ith nucleotide occurring at a locus in the sequence is p_i, then the average amount of information per nucleotide selected by the sender (or transmitted by the message source) is given by

$$H(X) = -K\sum_{i=1}^{n} p_i \log_2 p_i \quad (1)$$

where $H(X)$ is *Shannon's information measure*, also called *Shannon's entropy or information-theoretic entropy*, K is the proportionality constant usually taken to be unity, n is the number of nucleotides in the sequence, and $\log_2$ is the logarithm to the base of 2. $H(X)$ measures the before-the-fact uncertainty, i.e. the uncertainty as to which of the four nucleotides of the message source (i.e. sender in Table 1) will be selected for a locus in the nucleotide sequence under consideration. When the sequence is known, the uncertainty becomes zero and H now can be viewed as measuring the amount of information, I, that is associated with, or encoded in, the sequence (Wicken, 1987b; Yockey, 1992) transmitted to the receiver:

$$I = -\sum_{i=1}^{n} p_i \log 2p_i \quad (2)$$

Eqs. (1) and (2) seem redundant, but there are important physical processes underlying the transition from $H(X)$ to I, namely the processes (P) consisting of the *selection* of a message from the message source by the sender, *transmission* of the message through the communication channel, and the *reception* of the message by the receiver with the concomitant impact of the message on the receiver. We may represent this series of processes symbolically as follows:

$$H(X) \overset{P}{\rightarrow} I \quad (3)$$

To simplify the discussion, it was assumed that the efficiency of the communication channel (including the encoder and decoder; Shannon, 1948) is 100%, so that $H(X) = I$. Thus, Eq. (1) can be interpreted as representing the amount of the uncertainty *before the selection* of the message or the nucleotide sequence, while Eq. (2) can be interpreted as representing the amount of information transmitted by, or encoded in, the sequence recognized by the receiver *after reception*.

3. Energy

Living systems possess two basic forms of energies — *potential* and *kinetic*. *Potential energy* is the energy stored in a body or system as a consequence of its position, shape, or state (including gravitational energy, electrical energy, and chemical energy). *Kinetic energy* is the energy of motion, including the energies associated with the translational, rotational and vibrational motions of molecules, collectively called *thermal energies*.

Not all of the energies stored in living systems can be utilized to perform work due to the constraint imposed by the Second Law of thermodynamics (Callen, 1962). The fraction of the total energies of living systems that can perform work depends on the *mechanisms* or *machines* available, either macroscopic or microscopic, and the environmental conditions, such as temperature and pressure. The form of energy that provides the driving force for biological processes under constant temperature and pressure is *Gibbs free energy*, or often called simply *free energy*, symbolized by G (Callen, 1962; Lauffer, 1983; Tanford, 1983) and defined as:

$$G = H - TS \quad (4)$$

where H is enthalpy (or heat content, equal to the sum of the internal energy E and the pressure (P)–volume (V) work, PV), S is entropy, and T is the absolute temperature.

It was Schrödinger (1998) who first used the term 'negative entropy' in 1944 to indicate 'entropy with the negative sign,' namely, $-S$. 'Negative entropy' became the so-called '*negentropy*' in the hands of Brillouin (1962). If we designate negative entropy or negentropy with symbol N, Eq. (4) can be rewritten as:

$$G = H + TN \quad (5)$$

Schrödinger (1998) (p. 71) originally claimed that organisms fed on negative entropy. But he later recanted this claim after being criticized by F. Simon (Schrödinger, 1998, p. 74). As evident in Eq. (5), it is not N but G that drives life processes, in agreement with F. Simon.

Please note that there are two things that can be potentially quite confusing about Eqs. (1) and (4). First, the identical symbol, H, is used with two entirely unrelated meanings — in the senses of information-theoretic *entropy* in Eq. (1) and thermodynamic *enthalpy* in Eq. (4). Second, the identical sound value, '*entropy*,' is employed to designate two potentially unrelated physical entities — the *uncertainty* or *complexity* (Wicken, 1987a) of the message source in Eq. (1) and a measure of the disorder of a thermodynamic system in Eq. (4) (Schrödinger, 1998, p. 72).

There are two schools of thought regarding the relationship between Shannon entropy, H, in Eq. (1), and the thermodynamic entropy, S, in Eq. (4). The first group of scholars, including Shannon (Wicken, 1987a,b), Brillouin (1962), and more recent workers (Bennett, 1982; Stonier, 1996; Collier, 1999) posit that the information theoretic entropy and thermodynamic entropy are fundamentally related and quantitatively interconverted, leading to the conclusion that information-theoretic entropy can do work (Bennett, 1982). In contrast, the second group of scholars (Pierce, 1980; Wicken, 1987b; Ji, 1988; Yockey, 1992) maintains that these two entropies are fundamentally different and cannot be combined algebraically.

One of the evidences supporting the latter position is that the units of Shannon and thermodynamics entropies are different: Shannon's entropy H is expressed in *bits*, while thermodynamic entropy S has the unit of *calories/degree*. Therefore they cannot be directly (i.e. without any multiplication factors) combined algebraically. Another mundane evidence in favor of the second position is our common experience that the vast amount of information stored in a book cannot do any work, until and unless the book is coupled to, and hence influences, an energy-utilizing agent who can read the book and do work in accordance to the instructions written in it.

Because information is sometimes called or associated with *'negative entropy'* or '*negentropy*' (Brillouin, 1962; Collier, 1999), it is easy to make the mistake of associating information I in Eq. (2) with the '$-S$' term in Eq. (4) or with the N term in Eq. (5), thereby concluding that I can be absorbed into G and hence conclude that information can do work (Bennett, 1982; Collier, 1999). This conclusion would be judged wrong by the second school of thought mentioned above (Wicken, 1987b; Ji, 1988; Yockey, 1992), including the position taken in this paper.

4. Conformons in proteins

The idea that mechanical (i.e. *conformational*) properties of proteins play fundamental roles in enzymic catalysis dates back to at least the 1950s (Lumry, 1974; Lumry and Gregory, 1986). McClare (1971, 1974) and others (Ji, 1974b, 1991; Shaitan and Rubin, 1982; Welch and Kell, 1986) pursued this line of research under the rubric of '*molecular energy machines*' or more simply '*molecular machines*' — prefiguring the current interest in *molecular motors* and *single-molecule mechanics* (Sweeney, 1996; Noji et al., 1997). Implicit in the concept of *molecular machines* is the notion that proteins possess *potential energies* (necessary to generate internal mechanical forces) and *information* (to control the generation, annihilation, and direction of such forces). In 1974, this dual requirement of *energy* and *information* for enzymic catalysis was explicitly recognized in the formulation of the hypothesis that *enzymes provide not only energy* (in the form of conformational strains) *but also 'catalytic messages'* (in the form of local amino acid sequences constituting catalytic cavities) *in overcoming the activation free*

energy barriers during enzymic catalysis (Ji, 1974b, 1979).

By 1985, it became evident that conformons can serve as the source of both free energy and catalytic (or genetic) information that are necessary and sufficient for driving various functions of biopolymers. Thus, the concept of the conformon originally formulated by Green and Ji (1972) was extended to include *genetic information* in addition to *free energy*, leading to a new definition of conformons (Ji, 1985b,c) as '*genetically determined local conformational strains of biological macromolecules, each endowed with specific biological functions including ligand binding, catalysis, free energy storage, and free energy transfer,*' or as '*quanta of free energy and genetic information that underlie all biological actions at the molecular level.*'

Following the new definition of conformons, attempts were made to theoretically estimate the free energy and information contents of conformons in proteins. The free energy content of the conformon was approximated in the following manner. Since the amount of the free energy that conformons must transfer from redox reactions to ATP synthesis in mitochondria per coupling site was known, namely 16 kcal/mol (Slater, 1969), it was concluded that the free energy content of one conformon was in the range of 8–16 kcal/mol, assuming that the free energy transfer takes place through conformational changes in proteins in each coupling site of mitochondria in one or two major steps (Ji, 1990). It is common practice in biochemistry and bioenergetics that energy is expressed in the unit of *calories*. But this unit can be readily converted into ergs or joules (J) simply by using the relations, $1\ \text{erg} = 10^{-7}\ \text{J} = 2.3901 \times 10^{-8}$ cal (Moore, 1963).

The information content of the conformon was estimated in several ways:

1. If the number of different kinds of enzymes present in a unicellular organism is designated as N and the average number of the elementary steps catalyzed by an enzyme as e, then the total number of conformons in the cell must equal eN, if it is assumed that one conformon is required to drive one elementary step in enzymic catalysis (Ji, 1979, 1990, 1991; Ji and Finette, 1985). It appears reasonable to postulate that the maximum amount of information carried by eN conformons is approximately equal to the maximum amount of the genetic information encoded in the genome of the cell. The reasonableness of this postulate stems from the fact that the catalytic functions of enzymes are heritable from one generation to the next and hence must be encoded in the genome and most of the heritable properties of the cell require the participation of enzymes to be realized. Therefore, if we designate the total number of nucleotides in the genome as D and the maximum average information content of one conformon as $I_{\text{conformon}}$, then we can write the following relation:

$$I_{\text{conformon}} = (\log_2 4^D)/eN = 2D/eN$$

Using the estimated values of $N = 10^4$, $D = 10^7$ for a typical bacterial cell (Clark, 1977), and $e = 10$, $I_{\text{conformon}}$ can be calculated to be 200 bits (Ji and Finette, 1985).

2. To estimate the information content of conformons, a communication system was defined, consisting of the amino acid pool (with n different amino acids) as the message source, the biological evolution as the communication channel, and polypeptides (with an average number of amino acids $= m$) as the receiver (Ji, 1990). Then, the maximum information content of the average polypeptide ($I_{\text{polypeptide}}$) is:

$$I_{\text{polypeptide}} = \log_2 m^n \tag{7}$$

If each conformon consists of an alignment of x amino acid residues into a transient structure constituting the active site of an enzyme, the maximum information content of one conformon can be estimated to be:

$$I_{\text{conformon}} = \log_2[n!/(n-x)!] \tag{8}$$

If all of the information content of a polypeptide is transduced into the information content of p conformons, we have $I_{\text{polypeptide}} = pI_{\text{conformon}}$, which allows Eqs. (7) and (8) to be combined to give

$$[n!/(n-x)!]^p = m^n \tag{9}$$

Table 2
Information and free energy contents of conformons in proteins and DNA

	Proteins			DNA		
	Size (aa)[a]	Information (bits)	Free energy (kcal/N conformons)[b]	Size (bp)[c]	Information (bits)	Free energy (kcal/N conformons)[b]
Predicted[d,e]	~10	40–200	8–16	–	–	–
Measured[f]	–	–	–	100–300	200–600	500–2500

[a] The number of amino acid residues constituting one conformon.
[b] N is the Avogadro's number.
[c] The number of base pairs constituting one conformon.
[d] See Ji (1985a).
[e] See Ji (1990).
[f] From Table 3.

From the literature, we can make the following approximations: $n = 20$, $m = 150$, and $x = 10$ (Fersht, 1985). This leads to

$$[150!/(150-10)!]^p = 20^{150} \quad (10)$$

which is satisfied when $p = 9.02$, or approximately 9. Therefore, the maximum information content of one conformon is, $(\log_2 20^{150})/9 = 73$ bits.

3. If we postulate that the average number of amino acid residues constituting the active site during an elementary catalytic act is about 10, then Eq. (8) directly gives us an estimate of the information content of one conformon. This approach is tantamount to assuming that the biological evolution has allowed every amino acid residue in a polypeptide the equal opportunity to participate in active site construction: i.e. $I_{\text{conformon}} = \log_2[150!/(150-10)!] = \log_2 150!/140! = 72$ bits.
4. According to the information theory, it takes at least $0.693RT$ of energy to transmit one bit of information (Pierce, 1980). Therefore, if we designate the energy content of one conformon as $E_{\text{conformon}}$, then the maximum amount of information that one conformon can transmit is $E_{\text{conformon}}/0.693RT$ bits, or $(16 \times 10^3$ cal/mol)/(0.693)(1.987 cal/mol K)(300 K) = 38.7 bits.

Based on these estimates, we can conclude that the free energy and information contents of one conformon in proteins are approximately $8-16$ kcal/mol and 40–200 bits, respectively (Table 2).

5. Conformons in DNA

Although the concept of *conformons* was first invoked to account for the functional properties of proteins catalyzing oxidative phosphorylation in mitochondria (Green and Ji, 1972), the first direct experimental evidence for the existence of conformons emerged from the study of DNA. Weil and Vinograd (1963) reported electron microscopic evidence that circular DNA molecules existed in twisted conformations. The term '*supercoiling*' was introduced to indicate this topological state of covalently closed DNA duplexes (i.e. double helixes). Since supercoiled DNA duplexes contain conformational free energy (Bauer and Vinograd, 1970; Hsieh and Wang, 1975; Maxwell and Gellert, 1986) as evidenced by regional strand separations (Dean and Lebowitz, 1971; Beerman and Lebowitz, 1973), supercoiled DNA can be said to contain *conformons* as defined by Green and Ji (1972) and Ji (1985b,c).

The discovery of the enzymes that regulate the extent of supercoiling of DNA (Wang, 1971; Gellert et al., 1976) provided another experimental evidence for the presence of conformons in DNA. Numerous experimental observations have now established the concept that conformational or mechanical strains or stresses (i.e. *conformons*) play important roles in DNA functions (Wang, 1982, 1985, 1994, 1996; Gellert, 1981; Drlica, 1984), including DNA replication and transcription (Liu and Wang, 1987; Bramhill and Korn-

berg, 1988; Kowalski et al., 1988; Kowalski and Eddy, 1989; Natale et al., 1992; Miller and Kowalski, 1993; Drolet et al., 1994; Lin and Kowalski, 1994; Huang and Kowalski, 1996) and DNA premelting and drug intercalation (Banerjee and Sobell, 1983).

The topology of covalently closed, circular DNA duplexes is described by White's formula (White, 1969; White and Bauer, 1986):

$$Lk = Tw + Wr \tag{11}$$

where Lk is the *linking number*, namely the number of times one strand of the DNA double helix passes over the other if the molecule were constrained to lie on a plane; Tw is the total *twist*, the number of times either strand rotates around the central axis of the molecule; and Wr is the *writhing number* which measures the coiling of the DNA axis. In a covalently closed DNA duplex, Lk remains invariant even when the shape of the duplex changes due to bending, twisting, or local strand separation. Changes in Tw involve alterations of local helicity, whereas changes in Wr entail bending of the DNA duplex axis. In vivo, enzymes commonly maintain topological domains of DNA in negatively supercoiled states, in which their linking numbers Lk are smaller than their relaxed values, Lk_0. The resulting '*linking difference*' or '*linking deficiency*,' $\alpha = Lk - Lk_0 < 0$, must be accommodated by twisting and/or writhing deformations obeying the following conservation equation:

$$\alpha = \Delta Tw + \Delta Wr \tag{12}$$

where ΔTw and ΔWr indicate the excessive changes in twist and writhe, respectively, that are induced by linking deficiency, α. Therefore, the linking deficiency α can be viewed as a quantitative measure of the free energy content of conformons embedded in a supercoiled DNA duplex (Bauer and Vinograd, 1970; Depew and Wang, 1975; Hsieh and Wang, 1975; Pulleyblank et al., 1975; van Workum et al., 1996). For small values of α, we can associate at least 10 kcal/mol of free energy of conformational strains for each negative superhelical turn, because (1) one negative unit value of α is equivalent to removing one helical turn which causes the separation of 10.4 base pairs (Maxwell and Gellert, 1986), and (2) the average free energy required to separate one base pair in DNA oligomers (6–16 nucleotide long) has been found to be approximately 1.03 kcal/mol (the average G° value estimated from Table 2 in Breslauer et al., 1986). However, it should be kept in mind that, as the value of α increases, the free energy of superhelix formation increases rapidly due to the nonlinear dependence of the free energy on α (Bauer and Vinograd, 1970; Depew and Wang, 1975).

Eq. (12) provides a mechanism by which *changes in the secondary structure reflected in* ΔTw *can be coupled to those in the tertiary structure of DNA duplexes measured by* ΔWr. When supercoiling is introduced into a domain of covalently closed DNA duplex, some of the free energy of supercoiling can be localized at specific sites as *conformons*, or *SIDD* (see below), causing local regions to untwist or strand-separate fully or in part, leading to a relaxation of the rest of the domain by a corresponding amount (Benham, 1996a,b).

Benham (1992, 1993, 1996a,b) recently developed a statistical mechanics-based computer program that predicts the extent and location of what he termed '*stress-induced duplex destabilization* (SIDD)' in covalently closed DNA duplexes. Free energy is associated with each base pair in a covalently closed superhelical DNA duplex that depends on three factors: (1) the number and base compositions of unpaired regions, (2) the extent of inter-strand twisting experienced by these regions, and (3) the superhelical deformation (i.e. linking difference) of the DNA domain involved. If i_x indicates the conformational states in which the base pair at position x is separated, the ensemble average free energy of all such states is given by

$$\bar{G}(x) = \frac{\sum_{ix} G(i_x) \exp[-G(i_x)/RT]}{\sum_{ix} \exp[-G(i_x)/RT]} \tag{13}$$

where $G(i_x)$ indicates the Gibbs free energy of state i_x. Then the incremental free energy required to separate the base pair at position x is given by

Table 3
Stress-induced duplex destabilizations in DNA sequences

DNA sequence	Sequence length (bp)	Linking difference (turns)	Destabilized regions		
			Centered at sequence location	Length (bp)	$G_{\text{conformon}}$[a] (kcal/mol)
ColE1[b]	6646	−34	420	220	1760
			5080	230	2070
pBR322[b]	4363	−26	3220	225	2250
			4140	140	980
Polyoma virus strain a2[b]	5297	−30	530	160	800
			1510	180	1620
			2900	180	1800
			3460	150	450
			5030	220	660
Bacteriophage f1[b]	6407	−30	1600	180	540
			3160	200	1200
			3920	140	1120
			4360	220	2200
			4610	200	1000
			5900	210	2100
BPV-1[b]	7945	−37	2080	210	1680
			4070	260	1560
			7100	220	2200
Chicken histone H5 gene[c]	–	−27	360	90	810
		−35	4400	150	1500

[a] Calculated according to Eq. (16) based on data from figures in Benham (1993, 1996a).
[b] Benham (1993).
[c] Benham (1996a).

$$G(x) = \bar{G}(x) - \bar{G} \tag{14}$$

where G is the ensemble average free energy of the system and $\bar{G}(x)$ is the average free energy of all states i_x in which the base pair at position x is separated. The plot of $G(x)$ against x gives the so-called helix destabilization profile. Sites where separation is favored at equilibrium is characterized by $G(x) < 0$, while sites where separation is unfavorable have $G(x) > 0$. A region of helix destabilization appears in this plot as a location with a reduced $G(x)$ value. If we designate the extent of this destabilization as

$$\Delta G(x) = G(x') - G(x) \tag{15}$$

where x' indicates the position of the stabilized (i.e. Watson–Crick base paired) region nearest to x, and $G(x')$ is the incremental free energy needed to separate base pairs at x', then the free energy of the conformon, $G_{\text{conformon}}(l_x)$, associated with a destabilized region of length l_x centered at position x can be calculated from the following relation:

$$G_{\text{conformon}}(l_x) = \Sigma_{lx} \Delta G(x) \tag{16}$$

where the summation is from position $(x - l_x/2)$ to position $(x + l_x/2)$. Eq. (16) is significant because it indicates that SIDD and conformons are equivalent. Therefore, using Eq. (16) and the data obtained from the figures published by Benham (1993, 1996a,b), the free energy and information contents of DNA conformons can be estimated as shown in Table 3.

DNA conformons are localized in DNA domains 100–300 nucleotides long. From this it is

possible to estimate the maximum information contents associated with DNA conformons. These values range from 200 ($=\log_2 4^{100}$) to 600 ($=\log_2 4^{300}$) bits. The free energies associated with these conformons vary from 450 to 2250 kcal/mol (Table 2). Evidently, the information and free energy contents of conformons in DNA are in general larger than those associated with conformons in proteins (see Table 2).

There are several noteworthy features in Table 3:

1. The physical size (and hence information content) and free energy content of conformons in DNA vary independently; i.e. there is no apparent correlation between the information and free energy contents of conformons in DNA.
2. Both the position and size of conformons in DNA vary depending on the extent of the superhelical stresses imposed on DNA duplexes (see the last two rows in Table 3). For example, the conformon initially centered at the base pair position 360 in the chicken histone H5 gene is shifted to a new site centered at the base pair position 4400 when the superhelical stress is increased from $\alpha = -27$ to $\alpha = -35$ turns. This is one of the most unexpected findings revealed by Benham's computer simulation of superhelically stressed DNA and illustrates the general principle that the conformational behaviors (and presumably functional properties) of DNA duplexes are determined not only by *nucleotide sequences* (reflecting *local* properties) but also by *mechanical stresses* applied to them (affecting DNA duplexes *globally*). In view of the potential importance of this finding in understanding DNA properties and in recognition of Benham's seminal observation, it may be justified to refer to this finding as the *Benham phenomenon* or the *Benham effect* for convenience.

   The Benham phenomenon arises from the coupling between local and global properties of covalently closed DNA duplexes and implicates two distinct mechanisms: (1) the *global-to-local effects*, namely the global factors (e.g. superhelical stresses) affecting local properties (e.g. site of SIDD), and (2) the *local-to-global effects*, where local factors such as nucleotide sequences affect global properties. The superhelical stress-dependent redistribution of conformons observed in the chicken histone H5 gene represents an example of the *global-to-local effects*. One striking example of a *local-to-global effect* is illustrated by Benham's finding that the deletion of a 38 base-pair sequence from a 3 kb yeast genomic sequence completely abolished the localization of two conformons at 150 and 750 base pair positions and replaced these conformons with a set of over a dozen poorly resolved conformons distributed over the entire genomic sequence (Benham, 1996a).
3. Furthermore, Benham found what he termed a '*tripartite SIDD pattern*' where superhelically stressed yeast genes contained conformons predominantly in their 5′ and 3′ flanks, while excluding conformons from the associated coding regions. This observation establishes the non-random nature of SIDD distributions and the potential role of SIDD in the regulation of gene expression. In passing, it should be pointed out that the phenomenon of SIDD described by Benham supports the concept of DUE (*DNA Unwinding Element*) proposed by Kowalski and coworkers as a *cis*-acting component essential in the initiation of DNA replication (Kowalski and Eddy, 1989; Huang and Kowalski, 1993; Miller and Kowalski, 1993).

6. Conformons as the quanta of biological actions: a hypothesis

In analogy to the concept of the *quantum of action* invoked by Max Planck (1858–1947) in 1900 to account for black-body radiation which was later found to be implicated in other molecular and submolecular interactions in physics and chemistry, it may be useful to define the concept of *quanta of biological actions* (*QBA*) as the ultimate molecular mechanisms responsible for living processes on the molecular level. It is here postulated that conformons represent QBA. Conformons can serve as QBA because they possess not

Table 4
A classification of conformons according to their biological functions

Conformon class	Functions	References
1. *Anderson conformons*	Origin of life	Anderson, 1983, 1987
2. *Frauenfelder–Lumry conformons*	Thermal fluctuations(*Virtual* conformons)	Lumry and Gregory, 1986; Frauenfelder, 1987
3. *Volkenstein–Jencks conformons*	Substrate and product binding	Volkenstein, 1972, 1986; Kemeny and Goklany, 1973, 1974; Jencks, 1975, 1994; Whitty et al., 1995
4. *Franck–Condon conformons*	Transition-state ES complex formation	Ji, 1974a, 1979, 1985a, 1991; Shaitan and Rubin, 1982
5. *Green–Ji conformons*[a]	Free energy transfer without vibrational excitations	Ji, 1974b, 1979, 1985a, 1991
6. *Davydov conformons*	Free energy transfer through vibrationally excited covalent bonds (i.e. solitons)	Green and Ji, 1972; Davydov, 1973; Scott, 1985; Ji, 1985a
7. *Kehkls conformons*[b]	DNA replication and transcription, through the soliton mechanism	Englander et al., 1980; Sobell et al., 1982; Banerjee and Sobell, 1983; Sobell, 1985
8. *Benham–Kowalski –Kornberg conformons*[c]	DNA replication and transcription, without vibrational excitation	Benham, 1990, 1992, 1993, 1996a,b; Bramhill and Kornberg, 1988; Kowalski et al., 1988, 1989
9. *Klonowski–Klonowska conformons*	Timing in proteins	Klonowski and Klonowska, 1982
10. *Gedda conformons*	Timing in DNA	Gedda and Brenci, 1978

[a] This was previously called the *Green–Ji–Davydov conformons* (Ji, 1991). Since there are two distinct ways of storing free energy in biopolymers, depending on whether *vibrationally excited* covalent bonds are utilized or not, it would be useful to distinguish between two classes of conformons, one independent of and the other absolutely dependent on vibrational excitation of covalent bonds (see Section 7 for further details).

[b] *Kehkls* is an acronym derived from surname initials of the authors of the seminal paper, Englander et al. (1980).

[c] In his lecture on DNA replication in *E. coli* delivered at the Robert Wood Johnson Medical School in Piscataway on April 16, 1991, Dr Kornberg invoked a mechanistic concept similar to the conformon. In my letter to him on the same date, I pointed out this fact and referred him to literature references on the subject, which he gracefully acknowledged in a letter to me dated May 13, 1991. I take the liberty of using his name as a part of the *Benham–Kowalski–Kornberg conformon*, with the full knowledge that Dr Kornberg may or may not endorse this concept. The same applies to Drs Benham and Kowalski and, in fact, to all the authors whose names appear in Table 3.

only *free energy* (to drive molecular processes) but also *information* (to control such processes). The idea that the *duality of free energy and information* is necessary and sufficient for all molecular work processes in living systems may be viewed as a fundamental postulate in molecular biology, and, when it is proven to be valid, it may be referred to as the *Principle of the Dual Requirement of Free Energy and Information for Molecular Processes* in biology, or more briefly the *Conformon Principle of Molecular Biology*.

During the past two and a half decades, many investigators have invoked *conformons* or *conformon-like entities* to account for living processes on the molecular level, ranging from the origin of life to enzymic catalysis, and from oxidative phosphorylation to the timing of gene expression. In order to accommodate all these results coherently within one theoretical system, it is necessary to postulate the existence of a set of at least nine distinct classes of conformons, each class serving a unique set of biological functions as indicated in Table 4. One of the assumptions underlying Table 4 is that all solitons (if they exist) can be viewed as examples of conformons but not all conformons are solitons (see Section 7 for a justification of this generalization). As pointed out elsewhere (Ji, 1991), given these different kinds of conformons, most, if not all, mechanistic problems in molecular biology can be solved in principle. But

critical experimental data to evaluate this claim has yet to be obtained. Such a universality of conformons as mechanistic solutions to all aspects of living processes on the molecular level supports the postulate that conformons are *quanta of biological actions*.

7. Conformons and solitons: similarities and differences

It is important to make clear distinctions between two sets of closely related terms: (1) *conformational* (also called *noncovalent*) versus *configurational* (also called *covalent*) changes on the one hand, and (2) *electronic* versus *nuclear* rearrangements on the other. *Conformational* changes result from rotations of groups of atoms around covalent bonds, from bending of covalent bonds, or from the excitation of vibrational motions of covalent bonds (Davydov, 1973; Zgierski, 1975), all without breaking or forming any covalent bonds. In contrast, *configurational* changes always involve breaking and/or forming one or more covalent bonds. Good examples of configurational changes are provided by topological isomers of covalently closed circular DNA duplexes with different α values, interconversion between which requiring nicking and closing of DNA strands catalyzed by topoisomerases (Maxwell and Gellert, 1986). In contrast, conformational changes are exhibited by a topological isomer having a given value of α but existing in different *conformational states* characterized by different amounts of twisting (*Tw*) and writhing (*Wr*). These parameters must obey the conservation equation, $\alpha = \Delta Tw + \Delta Wr$. These different conformational states of DNA are called *conformational isomers*, or *conformers*. The interconversion among the different conformers of a DNA topoisomer can proceed without any enzyme-catalyzed breaking or forming of covalent bonds.

Conformational changes generally require activation free energies (ca 1–5 kcal/mol) much smaller than those required for configurational changes (ca 20–50 kcal/mol). In addition, during conformational changes, the motions of valence electrons and their associated nuclei are tightly coupled and confined within covalent bonds. However, during configurational changes, valence electrons and their associated core (i.e. nuclei + non-valence electrons) are forced apart beyond equilibrium distances, at least transiently. Because electronic motions take place much more rapidly than nuclear motions ($\sim 10^{-15}$ vs. $\sim 10^{-13}$ s; Moore, 1963), all electronic rearrangements accompanying configurational changes must be preceded by associated nuclear rearrangements. This requirement is known as the *Franck–Condon Principle*, first formulated in the field of inorganic electron transfer reactions (Reynolds and Lumry, 1966). This principle was generalized in 1974 (Ji, 1974b, 1979) to include not only electron transfer processes but also other physicochemical processes such as ligand binding to receptors and enzyme-catalyzed group transfer reactions, including the proton and the *phosphoron* (i.e. the reactive phosphoryl group, PO_3^-; Ji, 1974a) transfer reactions.

The *generalized Franck–Condon principle* can be described as follows. Let us consider an elementary physical or chemical process in which a particle (the electron, the proton, the phosphoron (Ji, 1974a), or higher atomic aggregates) denoted by the symbol ° is transferred from a donor A to acceptor B:

$$(A^\circ + B)_r \leftrightarrow (A + B^\circ)_p \tag{17}$$

where the parentheses denote the microenvironment (e.g. binding site or catalytic cavity geometries or conformations) of the *reactant* (*r*) or *product* (*p*) system. The generalized Franck–Condon principle states that, if the rate of the microenvironmental changes is significantly slower than the rate of the particle transfer (by a factor of about approx. 10^2), then Eq. (17) will proceed if and only if the microenvironmental change from the reactant state, $(\ )_r$, to the transition state, $(\ )^{\neq}$, precedes the particle transfer process $A^\circ B \rightarrow AB^\circ$: i.e.

$$(A^\circ + B)_r \leftrightarrow (A^\circ B)^{\neq} \leftrightarrow (AB^\circ)^{\neq} \leftrightarrow (A + B^\circ)_p \tag{18}$$

where the symbol, $(\ )^{\neq}$, represents the transition state. In analogy to the original Franck–Condon principle (Reynolds and Lumry, 1966), it is postulated here that the total free energy of the

reactants' activated complex, $(A^{\circ}B)^{\neq}$, is identical (within the limits of the Uncertainty Principle) with the total free energy of the products' activated complex, $(AB^{\circ})^{\neq}$, so that the particle can be associated with either A or B at the transition state (also called the *Franck–Condon state*) with an equal probability.

In Eq. (18), both *conformational* changes (as represented by the microenvironmental alterations from the 'r' state to the 'p' state) and *configurational* changes (i.e. the particle transfer from A to B) occur together in a time-coordinated manner in accordance with the restrictions imposed by the generalized Franck–Condon principle; namely, *conformational changes precede the configurational changes*. We can derive two important corollaries from the generalized Franck–Condon principle:

1. *Conformational changes can occur without any configurational changes.*
2. *Configurational or covalent changes cannot occur without requisite conformational changes preceding them.*

As will be shown below, Corollary 2 plays a critical role in differentiating *conformons* from *solitons*. In addition, this corollary provides a useful guide to formulating molecular mechanisms by which free energy of an exergonic chemical reaction may be stored in proteins as conformons (Ji, 1974a) (also see below).

One of the characteristics common to both *conformons* and *solitons* is their ability to store energy in biopolymers *mediated by conformational changes* (Ji, 1985a; Scott, 1985). Since both entities can carry useful energy, conformons and solitons are nearly identical in their ability to provide thermodynamic force for driving free energy-requiring molecular processes in living systems. However, there is one important difference between conformons and solitons. Detailed molecular mechanisms for transducing chemical free energy (e.g. the free energy of ATP hydrolysis) to *conformons* have been available in the literature for over two decades (Ji, 1974b, 1979), but, to the best knowledge of this author, no comparable molecular mechanisms have yet been proposed for generating *solitons* from exergonic chemical reactions.

In order for an enzyme to transduce the free energy of an exergonic chemical reaction into conformons, the enzyme must contain an *elastic element* (e.g. α-helix) that not only can exist in ground and energized conformational states but also is allosterically coupled to the active site geometry of the enzyme so that the mechanical state of the enzyme as a whole both affects and is influenced by the chemical reactions (i.e. electronic rearrangement) occurring at the catalytic site. The overall process of the chemical-to-conformational free energy transduction can be broken down into four major steps: *binding*, *activation*, *deactivation*, and *dissociation*:

a) *Binding*: $\alpha + \beta + \boldsymbol{E} \leftrightarrow \boldsymbol{E}^{\#}(\alpha\beta)$

b) *Activation*: $\boldsymbol{E}^{\#}(\alpha\beta) \leftrightarrow \boldsymbol{E}^{\neq}(\alpha\beta) < \text{- - } > \boldsymbol{E}^{\neq}(\gamma\delta)$

c) *Deactivation*: $\boldsymbol{E}^{\neq}(\alpha\beta) < \text{- - } > \boldsymbol{E}^{\neq}(\gamma\delta) \leftrightarrow \boldsymbol{E}^{\$}(\gamma\delta)$

d) *Dissociation*: $\boldsymbol{E}^{\$}(\gamma\delta) \leftrightarrow \boldsymbol{E}^{*} + \gamma + \delta$

$$\alpha + \beta + \boldsymbol{E} \leftrightarrow \boldsymbol{E}^{*} + \gamma + \delta \tag{19}$$

where $\boldsymbol{E}$ is an enzyme, superscript $^{\#}$ denotes a thermally induced conformational strain of the elastic element, which is also called a *virtual conformon* (see below). Superscript $^{\neq}$ indicates the conformationally strained transition state of the enzyme, superscript $^{\$}$ refers to a transient conformational state thermally accessible directly from conformationally energized state, $\boldsymbol{E}^{*}$ and not from the ground state conformational state, $\boldsymbol{E}$. The symbol * denotes the presence of conformons in proteins. The Greek letters symbolize the molecules that undergo electronic transitions catalyzed by $\boldsymbol{E}$: α and β are high-energy reactants, and γ and δ are low-energy products so that the reaction, $\alpha + \beta \rightarrow \gamma + \delta$, proceeds with a net decrease in the Gibbs free energy of the system. The double arrowed, dotted line indicates the transition (or Franck–Condon) state of the enzyme substrate complex, which is accessible from both the reactant and product systems, as required by the *principle of microscopic reversibility* (Frost and Pearson, 1961). The parentheses symbolize the active site of the enzyme. As evident in steps (b) and (c), the conformational transition, $\boldsymbol{E}^{\#} \leftrightarrow \boldsymbol{E}^{\neq}$ or $\boldsymbol{E}^{\$} \leftrightarrow \boldsymbol{E}^{\neq}$, preceded the configurational change, $\alpha\beta \rightarrow \gamma\delta$, or $\gamma\delta \rightarrow \alpha\beta$, in agreement with the gener-

alized Franck–Condon principle. The net result of Eq. (19) is that a part of the free energy released from the chemical reaction, $\alpha + \beta \rightarrow \gamma + \delta$ is stored in $\boldsymbol{E}^*$ as *conformons*. The free energy of conformons, $\Delta G_{\text{conformon}}$, must be only a fraction of the total free energy of the exergonic reaction, $\Delta G_{\text{reaction}}$:

$$G_{\text{conformon}} = \in \times G_{\text{reaction}} \tag{20}$$

where $\in$ represents the overall efficiency ($0 < \in < 1$) of the chemical-to-conformon free energy transduction.

In addition to the conformational strain mechanism of free energy storage and transfer described above which does not depend on any vibrational excitations of covalent bonds, there exists another possible mechanism by which the free energy derived from an exergonic chemical reaction is stored in proteins: the storage of energy in the form of *vibrational excitations* of covalent bonds. Vibrationally excited covalent bonds in proteins usually dissipate their energies rapidly before they can perform useful molecular work (Volkenstein, 1972; see General discussion on pp. 108–110, Green, 1974; Lomdahl and Kerr, 1985), except under a highly stringent circumstances wherein vibrational motions of covalent bonds are self-trapped in proteins to form what is known as *the soliton* (Davydov, 1973; Scott, 1985).

Thus, *solitons* require vibrational excitations of covalent bonds as the mode of energy storage and transport. In contrast, *conformons*, by their original definition as conformational strains carrying free energy, can be formed by any mechanisms — whether involving vibrational excitations of covalent bonds or not — as long as free energy can be stored in proteins long enough to do useful work. Hence, solitons can be viewed as representing one of the potential molecular mechanisms for producing conformons in biopolymers but conformon formation need not depend solely on the solitonic mechanism. In other words, conformons can be generated either by *a vibrational excitation mechanism* or *a conformational excitation mechanism* without requiring vibrational excitations of covalent bonds. For convenience, the conformons that depend on vibrational excitations of covalent bonds were referred to as *Davydov conformons* and those that do not as *Green–Ji conformons* (Table 4).

Once conformons are generated in biopolymers, there are again two ways of transferring the free energy of conformons from one locus to another within a biopolymer or from one biopolymer to another in protein complexes: (1) *the direct transfer mechanism* in which conformons migrate from one site to another through a biopolymer (e.g. mediated by vibrational excitations of peptide groups and hence by solitons) without thermalization (Green and Ji, 1972), and (2) *the indirect transfer mechanism* whereby the disappearance (or thermalization) of a conformon at one site is synchronized with the appearance of a conformon at another site (Ji, 1974a) (Eq. (19)), the two sites being located either within one biopolymer or distributed over two biopolymers. In the second mechanism, thermal fluctuations of biopolymers play an essential role in that biopolymers initially '*utilize*' thermal energy of the environment to generate '*virtual conformons*', which last long enough for a coupled exergonic reaction to be catalyzed, releasing free energy more than sufficient to 'pay back' the thermal energy 'borrowed' from the environment, thus avoiding the violation of the second Law of Thermodynamics (Ji, 1974b, 1979, 1991). We can represent these two mechanisms symbolically as follows:

1. *Direct Conformon Transfer Mechanism*:

$$\mathbf{A} + \mathbf{B}^* \rightarrow \mathbf{A}^* + \mathbf{B} \tag{21}$$

2. *Indirect Conformon Transfer Mechanism*:

$$\mathbf{A} + \mathit{Heat} \rightarrow \mathbf{A}^{\#} \tag{22}$$

$$\mathbf{A}^{\#} + \mathbf{B}^* \rightarrow \mathbf{A}^* + \mathbf{B} + \mathit{Heat} \tag{23}$$

$$\mathbf{A} + \mathbf{B}^* \rightarrow \mathbf{A}^* + \mathbf{B} \tag{24}$$

where **A** and **B** are catalytic sites (or domains) located within a biopolymer or in two separate biopolymers, the superscript symbols * and # represent '*real*' and '*virtual*' conformons, respectively. 'Virtual'' conformons are those conformational strains generated spontaneously as a result of thermal fluctuations of biopolymers and contain energies that cannot be used to drive any net molecular work functions due to the second law

of thermodynamics. However, as long as the lifetime of the virtual conformon $\mathbf{A}^{\#}$ is long enough to be stabilized by the dissipation of the real conformon in **B***, an effective free energy transfer (i.e. Eq. (24)) can be accomplished without violating the Second Law (Ji, 1974b, 1979, 1991).

Eq. (22) represents the so-called a thermal stroke (Ji, 1974a), and Eq. (23) can be viewed as a '*free energy-driven thermal energy-to-free energy transduction*' process. A direct experimental support for the existence of virtual conformons in DNA was provided by Depew and Wang (1975). When a sample of circular DNA molecules was treated with bovine pancreatic DNase I to cleave one strand and reannealed with *E. coli* ligase after a period of incubation, a set of about eight DNA topoisomers was found to be formed with different values of the topological winding number α. This indicates that a part of the circular DNA molecules at the single-strand state undergoes thermally driven supercoiling (i.e. form virtual conformons). These thermally-induced supercoiled states apparently last long enough for DNA ligase to act on them at the nicked site to reseal circular DNA duplexes, a part of the free energy released during the resealing reaction being utilized to convert virtual conformons to real conformons.

8. Conformon exchange between proteins and DNA

If conformons exist in both proteins and DNA, is there any evidence that these conformons can be exchanged between them? Probably the best experimental system to answer this question is to be found in DNA gyrase and other type II topoisomerases (Wang, 1971, 1982, 1985, 1996; Gellert, 1981). These enzymes play critical roles in DNA replication and transcription (Liu and Wang, 1978), and are targets of antibacterial and anticancer therapeutic agents (Drolet et al., 1994; Liu, 1994; Wang, 1994). The ultimate understanding of the molecular mechanisms underlying the action of these enzymes may require employing the conformon concept. The purpose of this section is to demonstrate that the general molecular mechanism of biological energy coupling developed for *oxidative phosphorylation*, *active transport*, and *muscle contraction* (Ji, 1974a) — all based on the conformon concept — can be extended to the mechanism of the action of *DNA gyrase*. As will become evident, from the viewpoint of the conformon theory, DNA gyrase is a conformon-driven mechanochemical energy transducer or a *conformon-driven* molecular motor (Sweeney, 1996).

A set of molecular mechanisms was formulated (Ji, 1974a), by which conformons can be generated in enzymes (treated as *molecular machines* or what is now more popularly known as *molecular motors*) from exergonic chemical reactions such as ATP hydrolysis and redox reactions (see Eq. (19)), transferred from one catalytic site to another (see Eq. (24)), and utilized by enzymes to perform molecular work processes, such as ATP synthesis, active transport, and muscle contraction. The conformon-mediated ATP-utilizing molecular processes are based on the ability of conformons to effect two fundamental molecular changes, namely (1) the *modulation of the binding affinity* of enzymes for their substrates — be they ions (including protons, Na^+, K^+, etc.), electrons, phosphorons (i.e. the phosphoryl group; Ji, 1974a), or thin filaments — and (2) the *vectorial transfer of these substrates* relative to the center of mass of the molecular machine. Following Stein et al. (1974), the former molecular change will be referred to as '*transformation*' (or alternatively '*transaffinitization*') and the latter as '*translocation*.' Stein et al. (1974) successfully utilized these two concepts to account for the action of the Na^+/K^+ ATPase in molecular terms.

More specifically, the conformon model of ATP-driven molecular processes include the following three basic steps: (a) *binding* between enzymes and their substrates (e.g. through electrostatic attraction made possible by newly generated charges in enzymes), (c) *translocation* of substrates (driven by conformons), and (c) *dissociation* of substrates (e.g. through charge annihilation). Notice that steps (a) and (c) involve *transformation*, while step (b) implicates vectorial movement, or *translocation*. Since all these steps are coupled and occur cyclically, namely, $a \rightarrow b \rightarrow$

c → a, etc., the cycle can operate against any gradients (e.g. ion, mechanical, or phosphorylation potential) so long as at least one of these three steps is driven by conformons. Which of the component steps are actually driven by conformons and which occur passively depends on the enzymes and coupled processes involved. Available evidence indicates that, during oxidative phosphorylation, conformons are utilized to dissociate ATP from, and 'attract' ADP and Pi to, the active site of the ATP synthetase (Boyer, 1993; Borman, 1994; Souid and Penefsky, 1995), thus implicating *conformon-driven transformation*, or, equivalently, '*energy-driven conformational conversion*' of binding sites (Borman, 1994). But in the case of the DNA gyrase (Berger and Wang, 1996), conformons may drive not only *transformation* but also *translocation* as well, as explained below.

DNA gyrase is a bacterial type II topoisomerase consisting of two A subunits (105 kDa) and two B subunits (95 kDa) and catalyzes the supercoiling reaction through a coordinated *strand breaking-passing-resealing process* (Liu and Wang, 1978; Morrison and Cozzarelli, 1981; Lindsley and Wang, 1993; Shen, 1994; Berger and Wang, 1996). Mechanistic and electron microscopic studies have revealed that the enzyme has a shape similar to a pair of scissors, with the A subunits acting as the cutting blades (cutting, passing, and resealing DNA double strands) and the B subunits serving as the handlers (powered by ATP hydrolysis). The fact that the free energy-releasing catalytic event (i.e. ATP hydrolysis) and the free energy-consuming process (e.g. DNA strand passing) occur in two different subunits (or domains in the case of the yeast DNA gyrase; Berger and Wang, 1996) strongly supports the notion that free energy released from ATP hydrolysis must be effectively transferred from one site to the other, even across an inter-subunit gap in the case of the bacterial enzyme (but not for the yeast enzyme). Such a free energy transfer process most likely occurs via the indirect conformon transfer mechanism rather than through the direct, soliton-mediated transfer mechanism.

Combining the conformon model of biological energy coupling (Ji, 1974a) and the *molecular-clamp* model of DNA gyrase (Berger and Wang, 1996), the following mechanism has been deduced for the mechanism of DNA supercoiling catalyzed by DNA gyrase. The main features of this mechanism may be applied generally to all other topoisomerases II:

$$\mathrm{ATP} + \mathbf{B}\cdot\mathbf{A}(\mathrm{G_c}) + \mathrm{T_u} \overset{1}{\leftrightarrow} (\mathrm{ATP})\mathbf{B}^{\#}\cdot\mathbf{A}(\mathrm{G_c}) + \mathrm{T_u}$$

$$(\mathrm{ATP})\mathbf{B}^{\#}\cdot\mathbf{A}(\mathrm{G_c}) + \mathrm{T_u} \overset{2}{\leftrightarrow} (\mathrm{ATP})\mathbf{B}\cdot\mathbf{A}^{\#}(\mathrm{G_c}) + \mathrm{T_u}$$

$$(\mathrm{ATP})\mathbf{B}\cdot\mathbf{A}^{\#}(\mathrm{G_c}) + \mathrm{T_u} \overset{3}{\leftrightarrow} (\mathrm{ATP})\mathbf{B}\cdot\mathbf{A}(\mathrm{G_o/T_u})$$

$$(\mathrm{ATP})\mathbf{B}\cdot\mathbf{A}(\mathrm{G_o/T_u}) \overset{4}{\leftrightarrow} (\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}^{*}\cdot\mathbf{A}(\mathrm{G_o/T_u})$$

$$(\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}^{*}\cdot\mathbf{A}(\mathrm{G_o/T_u}) \overset{5}{\leftrightarrow} (\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}\cdot\mathbf{A}^{*}(\mathrm{G_o/T_u})$$

$$(\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}\cdot\mathbf{A}^{*}(\mathrm{G_o/T_u}) \overset{6}{\leftrightarrow} (\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}\cdot\mathbf{A}^{\&}(\mathrm{G_o/T_u^{\$}})$$

$$(\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}\cdot\mathbf{A}^{\&}(\mathrm{G_o/T_u^{\$}}) \overset{7}{\leftrightarrow} (\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}\cdot\mathbf{A}^{\&}(\mathrm{T_d/G_o})$$

$$(\mathrm{ADP}\cdot\mathrm{Pi})\mathbf{B}\cdot\mathbf{A}^{\&}(\mathrm{T_d/G_o}) \overset{8}{\leftrightarrow} \mathrm{ADP} + \mathrm{Pi} + \mathrm{T_d} + \mathbf{B}\cdot\mathbf{A}(\mathrm{G_c})$$

$$\mathrm{ATP} + \mathrm{T_r} \overset{9}{\leftrightarrow} \mathrm{ADP} + \mathrm{Pi} + \mathrm{T_l}$$

where the bold letters **A** and **B** are the DNA gyrase subunits; the subscripts c (closed) and o (open) indicate the closed and open states of the G-segment, respectively; and subscripts u (up) and d (down) indicate the topological state of the *T*-segment before and after passing through the open *G*-segment, respectively, thereby changing the linking deficiency number α; and superscripts $^{\#}$, * and $^{\&}$ and designate different conformons stored in proteins; and superscript $^{\$}$ indicates conformons stored in DNA (i.e. the Benham–Kowalski–Kornberg conformon in Table 4).

To conveniently distinguish between different conformons involved in topoisomerase reactions, I am taking the liberty of naming these conformons as the Wang ($^{\#}$), Gellert (*), and Kowalski ($^{\&}$) conformons — with the full knowledge that these authors may or may not accept the validity of the conformon concept. It should be pointed out that the Wang and Kowalski conformons most likely belong to the family of the Volkenstein–Jencks conformons, while the Gellert conformon is best regarded as a member of the Green–Ji family of conformons. Clearly, the con-

formon stored in the T-segment (symbolized by $) is a member of the Benham–Kowalski–Kornberg family of conformons (Table 4). Please note that the name, Kowalski, appears in two different contexts, as part of the *family name* (the Benham–Kowalski–Kornberg conformon) and as a *given name*, so to speak, in the Kowalski conformon.

In step 1, a part of the free energy of binding of ATP to the **B** subunit of DNA gyrase already bound to the G-segment of DNA is stored as the Wang conformon in **B**. In step 2, the Wang conformon is transferred from the **B** to the **A** subunit, where it is used to (1) open the G-segment and bind the T-segment right of the G-segment (see step 3). In step 4, ATP is hydrolyzed and the free energy so generated is postulated to be stored in the **B** subunit as the Gellert conformon (symbolized by *), which is again transferred from the **B** to the **A** subunit in step 5. In step 6, a part of the Gellert conformon is transferred to the T-segment, thus forming a Benham conformon which is thought to perform the translocation of the T-segment from the right to the left side of the G-segment in step 7. The residual conformon in the **A** subunit (symbolized by superscript &) now causes the closing of the G-segment as well as the desorption of the T-segment, ADP and Pi from the enzyme (step 8). The net result of these series of steps is the movement of the T-segment through the G-segment, thus decreasing the linking number by one unit and translocation of the T-segment from right (r) to the left (l) relative to the G-segment (see step 9).

The eight-step mechanism described above is reminiscent of the eight-step conformon model of oxidative phosphorylation called the *Madisonator* proposed in 1974 (Ji, 1974a, 1976, 1991). Both these mechanisms can be viewed as self-organizing chemical reaction–diffusion systems, or dissipative structures (Prigogine, 1977, 1980) that perform specific functions driven by chemical reactions. Just as the function of mitochondria includes supplying ATP to the cell, so one of the major functions of topoisomerases II is to maintain and regulate the mechanical tensions of covalently closed DNA topological domains in the nucleus, thereby controlling various DNA functions. Since all self-organizing chemical reaction–diffusion systems are named after a city followed by the suffix '-ator' (e.g. the *Brusselator*, *Oregonator*, etc.), it is here recommended that the conformon model of DNA gyrase presented above be referred to as the *Bostonator* for convenience. It is hoped that the *Bostonator* will serve as a useful theoretical model of DNA gyrase that can lead to designing novel anticancer and antibacterial agents that can inhibit the enzyme selectively and efficiently in order to minimize their potential toxicities.

The fact that DNA gyrase is an enzyme capable of catalyzing the chemical-to-mechanical (i.e. ATP-to-DNA supercoiling) energy conversion is well-established. According to the *principle of microscopic reversibility* (Frost and Pearson, 1961), the reverse reaction must also occur, namely the conversion of *mechanical energy* (i.e. the energy stored in DNA supercoiling) into *chemical energy* of ATP, making DNA gyrase a *molecular mechanochemical energy transducer* or *converter*. In other words, the principle of microscopic reversibility predicts that the Bostonator shown above should be reversible starting from step 6, thereby converting the Benahm–Kowalski–Kornberg conformon (symbolized by superscript $) into a part of ATP. Such a mechanochemical energy conversion may have played an essential role in the origin of life (Ji, 1991) and may provide mechanisms for DNA to sense environmental conditions in the cytosol, consistent with the Bhopalator model of the living cell (Ji, 1991). The preliminary evidence recently obtained in our laboratory indicates that covalently closed, supercoiled pBR322 may indeed support the conversion of ADP and Pi into ATP (S. Ji and J. Lee, 1997, unpublished observation[1]).

[1] pBR322 plasmid (1 μg, covalently closed, supercoiled) incubated with DNA gyrase (Life Technologies) for 1–2 h in 35 mM Tris–HCl (pH 7.5), 20 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 2 mM spermidine-$(HCl)_3$, 0.1 μg/μl BSA, 10% (v/v) glycerol, 1 mM (K)phosphate (pH 7.5), 1 mM ADP, and 1 μl DNA gyrase (0.7 U), quenched by adding chloroform/isoamyl alcohol (24:1, v/v) and 2 μl 0.5 M EDTA (pH 8.0), centrifuged, and analyzed by agarose gel electrophoresis for 4 h at 75 V, indicated that the presence of ADP and Pi significantly decreased the rate of DNA gyrase-catalyzed unwinding of the supercoiled pBR322, presumably due to the formation of ATP driven by supercoils and the ATP so generated subsequently reintroducing supercoils in the same or different pBR322 molecules.

9. Conformons and the linguistics of DNA

There is theoretical and experimental evidence to support the hypothesis that the living cell uses a language very similar in principle to human language (Ji, 1997a,b). Living cells in multicellular organisms must communicate with one another in order for them to *survive* and *adapt*. Communication entails *transducing* and *transmitting* information through a communication channel mediated by *sign*s (Culler, 1986). Following the Swiss linguist F. de Saussure (1857–1913) who defined human language as "*a system of signs that represent ideas*," I proposed the definition of cell language as "*a self-organizing system of molecules, some of which encode, act as signs for, or trigger, gene-directed cell processes*" (Ji, 1997a,b).

The *molecule-based* cell language was found to be *isomorphic* with *sound-* and *visual signal-based* human language with respect to 10 out of the 13 design features of human language characterized by Hockett (1960). The isomorphism between cell and human languages justifies our transporting the fundamental concepts and principles developed in *linguistics* and *semiotics* (i.e. the science of signs) (Culler, 1986; Liszka, 1996) into molecular and cell biology.

Both cell and human languages can be treated as a six-tuple $\{L, W, S, G, P, M\}$, where L is the alphabet, W is a set of words (i.e. lexicon), S is an arbitrary set of sentences, G is the grammar, P is a set of physical mechanisms realizing a language (which includes mechanisms of phonation and audition), and M is a set of objects or events referred to by words and sentences. For more detailed discussions on the *isomorphism* between cell and human languages, readers are referred to the original articles (Ji, 1997a,b).

More recently, the cell language theory was applied to the analysis of the DNA structure and function, leading to the conclusion that there are three distinct classes of information encoded in DNA: (1) the *lexical* (i.e. structural genes), (2) *syntactic* (i.e. the physicochemical properties of DNA double helix), and (3) *semantic information* (probably encoded in the non-coding regions of DNA) (Ji, 1999a). To retrieve these various kinds of genetic information, it was postulated that DNA utilizes conformons whose information and free energy contents make it possible to express select genes at right times and for right durations, resulting in the creation of various intracellular dissipative structures (IDSs) (e.g. ion gradients). IDSs in turn are thought to drive all cell functions and thus represent the meaning or semantics of the DNA language (Ji, 1991, 1997a,b, 1999a). Such a role of conformons in cell language would be similar to the role of speech sounds in human language, since without speech sounds it would be difficult, if not impossible, to transfer information from a book or from the speaker's brain to another human being. So, it may be logical to conclude that

> "Conformons are to cell language what speech sounds are to human language."

10. Predictions

The concept of *conformons*, first introduced by various investigators more than two decades ago, can now be reasonably said to have been confirmed by experimental observations. If conformons are real, they should lead to predictions that can be tested by experiments. The following is a list of some of such predictions:

1. There may exists a finite set of different conformons (10^5–10^6?) in DNA, RNA, and proteins, that are necessary and sufficient to account for all living processes on the molecular level. Reminiscent of the periodic table in chemistry, these conformons may be organized into a set of families (10–20?) based on their distinct biological functions, each family having many members (10^3–10^5?).
2. Conformons, and not their components, either structural *information* (e.g. nucleotide sequence information in DNA) or *free energy* content

(e.g. linking number deficiency in supercoiled DNA), will be found to be responsible for controlling molecular processes in the cell. To the extent that the *information* and *energy* contents of conformons cannot be simultaneously measured experimentally, the ultimate molecular mechanisms underlying living processes cannot be unambiguously determined experimentally and thus will be associated with irreducible *uncertainties* or *fuzziness*.

3. It may be possible to establish *the law of conservation of conformons* in analogy to the law of conservation of energy in physics. The law of conservation of conformons (LCC) states that the function of *conformons* has been conserved throughout biological evolution — from the origin of life to the maintenance of life (Ji, 1991). LCC is consistent with the *principle of equivalence* (Table 1), according to which the generation of context-dependent information always depends upon the prior existence of an equivalent amount of information (Küppers, 1996).
4. Covalently closed circular DNA duplexes can undergo an increase in negative supercoiling upon lowering temperature (Hsieh and Wang, 1975). The conformational free energy so generated in supercoiled DNA may be converted into the free energy of ATP by treating the DNA molecule with topoisomerase II in the presence of ADP and Pi. If the 'spent' DNA is isolated and treated with single strand-specific DNase before raising temperature and re-annealing the molecule with DNA ligase (Depew and Wang, 1975), the cycle of conformon-to-ATP energy conversion may be repeated, thus giving rise to what may be called a *DNA-mediated thermal-to-chemical energy converter*. A successful construction of such a molecular energy converter would (1) further support the conformon hypothesis of biological energy transduction (Ji, 1974a), and (2) strengthen the validity of *the Princetonator*, a molecular model of the origin of life based on nucleic acid-mediated *thermal-to-chemical energy conversion* (Ji, 1991).
5. The chemiosmotic hypothesis of oxidative phosphorylation (Nicholls, 1982) will be found inadequate to account for the results from *single-molecule mechanical experiments* indicating that myosin molecules can store ATP free energy in the form of conformational strains (Service, 1997) and for the phenomenon of *mitochondrial control of apoptosis* universally requiring membrane depolarization (Kroemer et al., 1997). Chemiosmotic hypothesis of biological energy transduction explains the phenomenology of transmembrane proton movement associated with ATP hydrolysis or synthesis but does not adequately address in molecular terms the fundamental mechanisms for generating or utilizing transmembrane proton gradients. The conformon hypothesis provides such needed molecular mechanisms (Ji, 1974a, 1976, 1977, 1979).

11. Conclusions

Although definitive experimental proofs are still lacking, the concept of conformons has gained substantial experimental and theoretical support during the past 25 years (Green and Ji, 1972; Volkenstein, 1972; Davydov, 1973; Kemeny and Goklany, 1973). The conformon theory of biological energy transduction not only provides a coherent theoretical framework to account for a wide range of molecular biological processes in the living cell — from the origin of life to enzymic catalysis to gene expression — but also can generate testable predictions. The clearest evidence obtained so far in support of *conformons* is provided by the phenomenon of SIDD (strain-induced duplex destabilization) discovered by Benham (1996a,b). SIDD demonstrates the inseparable role of free energy and genetic information (embodied in conformons) in controlling gene expression. The conformon concept coupled with the theory of cell language (Ji, 1997a,b) and the linguistics of DNA (Ji, 1999a) may contribute importantly to the final unravelling of the structure and function of DNA, including the human genome.

References

Aczél, J., Daróczy, Z., 1975. On Measures of Information and their Characterizations. Academic Press, New York.

Anderson, P.W., 1983. Suggested model for prebiotic evolution: the use of chaos. Proc. Natl. Acad. Sci. USA 80, 3386–3390.

Anderson, P.W., 1987. Computer modeling of prebiotic evolution: general theoretical ideas on the origin of biological information. Comm. Mol. Cell. Biophys. 4, 99–108.

Banerjee, A., Sobell, H.M., 1983. Presence of nonlinear excitations in DNA structure and their relationship to DNA premelting and drug intercalation. J. Biomol. Struct. Dyn. 1, 253–262.

Bauer, W., Vinograd, J., 1970. Interaction of closed circular DNA with intercalative dyes II. The free energy of superhelix formation in SV40 DNA. J. Mol. Biol. 47, 419–435.

Baverstock, K.F., Cundall, R.B., 1988. Solitons and energy transfer in DNA. Nature 332, 312–313.

Beerman, T.A., Lebowitz, J., 1973. Further analysis of the altered secondary structure of superhelical DNA. Sensitivity to methyl mercuric hydroxide as chemical probe for unpaired bases. J. Mol. Biol. 79, 451.

Benham, C.J., 1990. Theoretical analysis of heteropolymeric transitions in superhelical DNA molecules of specified sequences. J. Chem. Phys. 92, 6294–6305.

Benham, C.J., 1992. Energetics of the strand separation transition in superhelical DNA. J. Mol. Biol. 225, 835–847.

Benham, C.J., 1993. Sites of predicted stress-induced DNA duplex destabilization occur preferentially at regulatory sites. Proc. Natl. Acad. Sci. USA 90, 2999–3003.

Benham, C.J., 1996a. Duplex destabilization in superhelical DNA is predicted to occur at specific transcriptional regulatory regions. J. Mol. Biol. 255, 425–434.

Benham, C.J., 1996b. Computation of DNA structural variability — a new predictor of DNA regulatory regions. CABIOS 12 (5), 375–381.

Bennett, C.H., 1982. Thermodynamics of computation — a review. Int. J. Theor. Phys. 21 (12), 905–940.

Berger, J.M., Wang, J.C., 1996. Recent developments in DNA topoisomerases II structure and mechanism. Curr. Opin. Struct. Biol. 6 (1), 84–90.

Bohm, D., 1979. Quantum Theory. Dover, New York.

Borman, S., 1994. High-resolution structure obtained for ATP synthesis enzyme. C and E News, September, pp. 31–32.

Boyer, P.D., 1993. The binding change mechanism for ATP synthetase — Some probabilities and possibilities. Biochim. Biophys. Acta 1140, 215–250.

Bramhill, D., Kornberg, A., 1988. Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the E. coli chromosome. Cell 52, 743–755.

Breslauer, K.J., Frank, R., Blöcker, H., Marky, L.A., 1986. Predicting DNA duplex stability from the base sequence. Proc. Natl. Acad. Sci. USA 83, 3746–3750.

Brillouin, L., 1962. Science and Information Theory, 2nd edn. Academic Press, New York.

Callen, H.B., 1962. Thermodynamics: An Introduction to the Physical Theories of Equilibrium Thermostatics and Irreversible Thermodynamics. Wiley, New York, pp. 90–106.

Careri, G., Wyman, J., 1984. Soliton-assisted unidirectional circulation in a biochemical cycle. Proc. Natl. Acad. Sci. 81, 4386–4388.

Careri, G., Wyman, J., 1985. Unidirectional circulation in a prebiotic photochemical cycle. Proc. Natl. Acad. Sci. 82, 4115–4116.

Ciblis, P., Cosic, I., 1997. The possibility of soliton/exciton transfer in proteins. J. Theor. Biol. 184, 331–338.

Clark, B.F.C., 1977. The Genetic Code. The Camelot Press, Southampton, p. 6.

Collier, J., 1999. Causation is the transfer of information. In: Sankey, H. (Ed.), Causation, Natural Laws and Explanation. Kluwer, Dordrecht.

Culler, J., 1986. Ferdinand de Saussure, Revised edn. Cornell University Press, Ithaca, NY.

Davydov, A.S., 1973. The theory of contraction of proteins under their excitation. J. Theor. Biol. 38, 559–569.

Davydov, A.S., 1987. Excitons and solitons in molecular systems. Int. Rev. Cytol. 106, 183–225.

Dean, W.W., Lebowitz, J., 1971. Partial alteration of secondary structure in native superhelical DNA. Nat. New Biol. 231, 5–8.

Depew, R.E., Wang, J.C., 1975. Conformational fluctuations of DNA helix. Proc. Natl. Acad. Sci. USA 72, 4275–4279.

Drlica, K., 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. 48 (4), 273–289.

Drolet, M., Wu, H.-Y., Liu, L.F., 1994. Roles of DNA topoisomerases in transcription. Adv. Pharmacol. 29A, 135–146.

Englander, S.W., Kallenbach, N.R., Heeger, A.J., Krumhansl, J.A., Litwin, S., 1980. Nature of the open state in long polynucleotide double helices: Possibility of soliton excitation. Proc. Natl. Acad. Sci. USA 77 (12), 7222–7226.

Fersht, A., 1985. Enzyme Structure and Mechanisms, 2nd edn. W.H. Freeman, San Francisco, CA.

Frauenfelder, H., 1987. Function and dynamics of myoglobin. Ann. N.Y. Acad. Sci. 504, 151–167.

Freund, J.A., 1996. Information, long-range correlations and extended memory of symbol sequences. In: Kornwachs, K., Jacoby, K. (Eds.), Information: New Questions to a Multidisciplinary Concept. Akademie-Verlag, Berlin, pp. 41–62.

Frost, A.A., Pearson, R.G., 1961. Kinetics and Mechanism, 2nd edn. Wiley, New York, pp. 211–213.

Gedda, L., Brenci, G., 1978. Chronogenetics: The Inheritance of Biological Time. Thomas, Springfield, IL.

Gellert, M., 1981. DNA topoisomerases. Annu. Rev. Biochem. 50, 879–910.

Gellert, M., Mizuuchi, K., O'Dea, M.H., Nash, H., 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA 73, 3872–3876.

Gernert, D., 1996. Pragmatic information as a unifying concept. In: Kornwachs, K., Jacoby, K. (Eds.), Information: New Questions to a Multidisciplinary Concept. Akademie-Verlag, Berlin, pp. 147–162.

Green, D.E., 1974. General discussion. Ann. N.Y. Acad. Sci. 227, 108–110.

Green, D.E., Ji, S., 1972. The electromechanochemical model of mitochondrial structure and function. In: Schultz, J., Cameron, B.F. (Eds.), Molecular Basis of Electron Transport. Academic Press, New York, pp. 1–44.

Hockett, C.F., 1960. The origin of speech. Sci. Am. 203 (3), 89–96.

Hsieh, T., Wang, J.C., 1975. Thermodynamic properties of superhelical DNAs. Biochemistry 14 (3), 527–535.

Huang, R.-Y., Kowalski, D., 1993. A DNA unwinding element and an ARS consensus comprise a replication origin within a yeast chromosome. EMBO J. 12 (12), 4521–4531.

Huang, R.-Y., Kowalski, D., 1996. Multiple DNA elements in ARS305 determine replication origin activity in a yeast chromosome. Nucleic Acids Res. 24 (5), 816–823.

Jencks, W.P., 1975. Binding energy, specificity, and enzymic catalysis: The Circe Effect. Adv. Enzymol. 43, 219–410.

Jencks, W.P., 1994. Reaction mechanisms, catalysis, and movement. Protein Sci. 3, 2459–2464.

Ji, S., 1974a. A general theory of ATP synthesis and utilization. Ann. N.Y. Acad. Sci. 227, 211–226.

Ji, S., 1974b. Energy and negentropy in enzymic catalysis. Ann. N.Y. Acad. Sci. 227, 419–437.

Ji, S., 1976. A model of oxidative phosphorylation that accommodates the chemical intermediate, chemiosmotic, localized proton and conformational hypotheses. J. Theor. Biol. 59, 319–330.

Ji, S., 1977. A possible molecular mechanisms of free energy transfer in oxidative phosphorylation. J. Theor. Biol. 68, 607–612.

Ji, S., 1979. The principles of ligand–protein interactions and their application to the mechanism of oxidative phosphorylation. In: Yagi, K. (Ed.), Structure and Function of Biomembranes. Japan Scientific Societies Press, Tokyo, pp. 25–37.

Ji, S., 1985a. Conformons and solitons: new concepts in bioenergetics. In: Mishra, R.K. (Ed.), The Living State — II. World Scientific Publishing Company, Singapore, pp. 563–574.

Ji, S., 1985b. The Bhopalator: a molecular model of the living cell. Asian J. Exp. Sci. 1, 1–33.

Ji, S., 1985c. The Bhopalator — a molecular model of the living cell based on the concepts of conformons and dissipative structures. J. Theor. Biol. 116, 399–426.

Ji, S., 1988. Watson–Crick and Prigoginian forms of genetic information. J. Theor. Biol. 130, 239–245.

Ji, S., 1990. The Bhopalator — A molecular model of the living cell: new developments. In: Mishra, R.K. (Ed.), Molecular and Biological Physics of Living Systems. Kluwer, Dordrecht, pp. 187–214.

Ji, S., 1991. Biocybernetics: A machine theory of biology. In: Ji, S. (Ed.), Molecular Theories of Cell Life and Death. The Rutgers University Press, New Brunswick, pp. 1–237.

Ji, S., 1997a. A cell linguistic analysis of apoptosis. Comm. Toxicol. 5 (6), 571–585.

Ji, S., 1997b. Isomorphism between cell and human languages: molecular biological, bioinformatic and linguistic implications. BioSystems 44, 17–39.

Ji, S., 1999a. The linguistics of DNA: Words, sentences, grammar, phonetics and semantics. Ann. N.Y. Acad. Sci. 870, 411–417.

Ji, S., 1999b. The cell as the smallest DNA-based molecular computer. BioSystems 52, 122–123.

Ji, S., Finette, S., 1985. Codons, conformons and dissipative structures: A molecular theory of the living state. In: Mishra, R.K. (Ed.), The Living State — II. World Scientific Publishing, Singapore, pp. 546–562.

Kauzmann, W., 1957. Quantum Chemistry: An Introduction. Academic Press, New York.

Kemeny, G., Goklany, I.M., 1973. Polarons and conformons. J. Theor. Biol. 40, 107–123.

Kemeny, G., Goklany, I.M., 1974. Quantum mechanical model for conformons. J. Theor. Biol. 48, 23–38.

Klonowski, W., Klonowska, M.T., 1982. Biophysical time on macromolecular level. Biomathematics 80, 28–33.

Kornwachs, K., Jacoby, K., 1996. Information: New Questions to a Multidisciplinary Concept. Akademie-Verlag, Berlin.

Kowalski, D., Eddy, M.J., 1989. The DNA unwinding element: a novel, *cis*-acting component that facilitates opening of the *Escherichia coli* replication origin. EMBO J. 8 (13), 4335–4344.

Kowalski, D., Natale, D.A., Eddy, M.J., 1988. Stable DNA unwinding, not 'breathing,' accounts for single-strand-specific nuclease hypersensitivity of specific A + T-rich sequences. Proc. Natl. Acad. Sci. USA 85, 9464–9468.

Kroemer, G., Zamzami, N., Susin, S.A., 1997. Mitochondrial control of apoptosis. Immunol. Today 18 (1), 44–51.

Küppers, B.-O., 1996. The context-dependence of biological information. In: Kornwachs, K., Jacoby, K. (Eds.), Information: New Questions to a Multidisciplinary Concept. Akademie-Verlag, Berlin, pp. 137–145.

Lauffer, M.A., 1983. The significance of entropy-driven processes in biological systems. Comm. Mol. Cell. Biophys. 2 (2), 99–109.

Lin, S., Kowalski, D., 1994. DNA helical instability facilitates initiation at the SV40 replication origin. J. Mol. Biol. 235, 496–507.

Lindsley, J.E., Wang, J.C., 1993. Study of allosteric communication between protomers by immunotagging. Nature 361, 749–750.

Liszka, J.J., 1996. A General Introduction to the Semeiotic of Charles Sanders Peirce. Indiana University Press, Bloomington, IN.

Liu, L.F., 1994. Preface. Adv. Pharmacol. 29a, xv.

Liu, L.F., Wang, J.C., 1978. *Micrococcus luteus* DNA gyrase: Active components and a model for its supercoiling of DNA. Proc. Natl. Acad. Sci. USA 75 (5), 2098–2102.

Liu, L.F., Wang, J.C., 1987. Supercoiling of the DNA template during transcription. Proc. Natl. Acad. Sci. USA 84, 7024–7027.

Lomdahl, P.S., Kerr, W.C., 1985. Do Davydov solitons exist at 300 K? Phys. Rev. Lett. 55 (11), 1235–1238.

Lomdahl, P.S., Layne, S.P., Bigio, I.J., 1984. Solitons in biology. Los Alamos Sci. 10, 2–22.

Lumry, R., 1974. Conformational mechanisms for free energy transduction in protein systems: Old ideas and new facts. Ann. N. Y. Acad. Sci. 227, 46–73.

Lumry, R., Gregory, R.B., 1986. Free-energy management in protein reactions: concepts, complications, and compensation. In: Welch, G.R. (Ed.) The Fluctuating Enzyme, John Wiley & Sons, New York, pp. 1–190.

Mahler, G., 1996. Quantum information. In: Kornwachs, K., Jacoby, K. Jr (Eds.), Information: New Questions to a Multidisciplinary Concept. Akademie-Verlag, Berlin, pp. 103–118.

Maxwell, A., Gellert, M., 1986. Mechanistic aspects of DNA topoisomerases. Adv. Protein Chem. 38, 69–107.

McClare, C.W.F., 1971. Chemical machines, Maxwell's demon and living organisms. J. Theor. Biol. 30, 1–34.

McClare, C.W.F., 1974. Resonance in bioenergetics. Ann. N.Y. Acad. Sci. 227, 74–97.

Miller, C.A., Kowalski, D., 1993. *cis*-acting components in the replication origin from ribosomal DNA of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 13 (9), 5360–5369.

Moore, W.J., 1963. Physical Chemistry, 3rd edn. Prentice-Hall, Englewood Cliffs, NJ, p. 597.

Morrison, A., Cozzarelli, N.R., 1981. Contacts between DNA gyrase and its binding site on DNA: Features of symmetry and asymmetry revealed by protection from nucleases. Proc. Natl. Acad. Sci. USA 78, 1416–1420.

Natale, D.A., Schubert, A.E., Kowalski, D., 1992. DNA helical stability accounts for mutational defects in a yeast replication origin. Proc. Natl. Acad. Sci. USA 89, 2654–2658.

Nicholls, D.G., 1982. Bioenergetics: An Introduction to the Chemiosmotic Theory. Academic Press, New York.

Noji, H., Yasuda, R., Yoshida, M., Kinoshita, K. Jr, 1997. Direct observation of the rotation of F_1-ATPase. Nature 386, 299–302.

Pierce, J.R., 1980. An Introduction to Information Theory: Symbols, Signals and Noise, 2nd edn. Dover, New York.

Pilar, F.L., 1968. Elementary Quantum Chemistry. McGraw-Hill, New York.

Polozov, R.V., Yakushevich, L.V., 1988. Nonlinear waves in DNA and regulation of transcription. J. Theor. Biol. 130, 423–430.

Prigogine, I., 1977. Dissipative structures and biological order. Adv. Biol. Med. Phys. 16, 99–113.

Prigogine, I., 1980. From Being to Becoming: Time and Complexity in Physical Sciences. W.H. Freeman, San Francisco, CA.

Pulleyblank, D.E., Shive, M., Tang, D., Vinograd, J., Vosberg, H.-P., 1975. Action of nicking-closing enzyme on supercoiled and nonsupercoiled closed circular DNA: Formation of a Boltzmann distribution of topological isomers. Proc. Natl. Acad. Sci. USA 72, 4280–4284.

Rebbi, C., 1979. Solitons. Sci. Am. 240 (2), 92–116.

Reynolds, W.L., Lumry, R.W., 1966. Mechanisms of Electron Transfer. Ronald Press, New York.

Salthe, S.N., 1996. Development and Evolution: Complexity and Change in Biology. MIT Press, Cambridge, MA.

Scott, A.C., 1985. Solitons in biological molecules. Comm. Mol. Cell. Biophys. 3 (1), 15–37.

Scott, A.C., Chu, F.Y.F., McLaughlin, D.W., 1973. The soliton: A new concept in applied science. Proc. IEEE 61 (10), 1443–1483.

Schiff, L.I., 1968. Quantum Mechanics, 3rd edn. McGraw-Hill, New York.

Schrödinger, E., 1998. What is Life? With Mind and Matter and Autobiographical Sketches. Cambridge University Press, Cambridge.

Service, R.F., 1997. Chemists explore the power of one. Science 276, 1027–1029.

Shaitan, K.V., Rubin, A.B., 1982. Equation of motion of conformon and primitive molecular machines for electron (ion) transport in biological systems. Mol. Biol. 16 (5), 1004–1018.

Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 5–18.

Shen, L.L., 1994. Molecular mechanisms of DNA gyrase inhibition by quinolone antibacterials. Adv. Pharmacol. 29A, 285–304.

Shen, Y.R., 1997. Solitons made simple. Science 276, 1520–1541.

Slater, E.C., 1969. The oxidative phosphorylation potential. In: Papa, S., Tager, J.M., Quagliariello, E., Slater, E.C. (Eds.), The Energy Level and Metabolic Control in Mitochondria. Adriatica Editrice, Bari, pp. 255–259.

Sobell, H.M., 1985. Actinomycin and DNA transcription. Proc. Natl. Acad. Sci. USA 82, 5328–5331.

Sobell, H.M., Banerjee, A., Lozansky, E.D., Zhou, G.-P., Chou, K.-C., 1982. The role of low frequency (acoustic) phonons in determining the premelting and melting behaviours of DNA. Comm. J. Mol. Sci. (Wuhan, China) 2 (4), 113–128.

Souid, A.-K., Penefsky, H.S., 1995. Energetics of ATP dissociation from the mitochondrial ATPase during oxidative phosphorylation. J. Biol. Chem. 270 (16), 9074–9082.

Stein, W.D., Eilam, Y., Lieb, W.R., 1974. Active transport of cations across biological membranes. Ann. N.Y. Acad. Sci. 227, 328–336.

Stonier, T., 1990. Information and the Internal Structure of the Universe. Springer, London.

Stonier, T., 1996. Information as a basic property of the universe. BioSystems 38, 135–140.

Sweeney, H.L., 1996. Special topic: Molecular motors of eukaryotic cells. Annu. Rev. Physiol. 58, 670.

Tanford, C., 1983. Mechanism of free energy coupling in active transport. Annu. Rev. Biochem. 52, 379–409.

van der Lubbe, J.C.A., 1997. Information Theory. Cambridge University Press, Cambridge.

Volkenstein, M.V., 1972. The Conformon. J. Theor. Biol. 34, 193–195.

Volkenstein, M.V., 1981. Simple physical presentation of enzymic catalysis. J. Theor. Biol. 89, 45–51.

Volkenstein, M.V., 1986. Electronic-conformational interaction in biopolymers. In: Welch, G.R. (Ed.), The Fluctuating Enzyme, John Wiley & Sons, New York, pp. 403–419.

Volkenstein, M.V., Dogonatze, R.R., Madumarov, A.K., Urushadze, Z.D., Kharkatz, Y.I., 1972. The theory of enzyme catalysis. Mol. Biol. 6 (3), 431–439.

Wang, J.C., 1971. Interaction between DNA and an *Escherichia coli* protein. J. Mol. Biol. 55, 523–533.

Wang, J.C., 1982. DNA topoisomerases. Sci. Am. 247 (1), 94–97.

Wang, J.C., 1985. DNA topoisomerases. Annu. Rev. Biochem. 54, 665–697.

Wang, J.C., 1994. DNA topoisomerases as targets of therapeutics: An overview. Adv. Pharmacol. 29A, 1–19.

Wang, J.C., 1996. DNA topoisomerases. Annu. Rev. Biochem. 65, 635–692.

Weil, R., Vinograd, J., 1963. The cyclic helix and cyclic coil forms of polyoma viral DNA. Proc. Natl. Acad. Sci. USA 50, 730–738.

Welch, G.R., Kell, D.B., 1986. Not just catalysts — Molecular machines in bioenergetics. In: Welch, G.R. (Ed.), The Fluctuating Enzyme, John Wiley & Sons, New York, pp. 451–492.

White, J.H., 1969. Self-linking and the Gauss integral in higher dimensions. Am. J. Math. 91, 693–728.

White, J.H., Bauer, W.R., 1986. Calculation of the twist and the writhe for representative models of DNA. J. Mol. Biol. 189, 329–341.

Whitty, A., Fierke, C.A., Jencks, W.P., 1995. Role of binding energy with coenzyme A in catalysis by 3-oxoacid coenzyme A transferase. Biochemistry 34, 11678–11689.

Wicken, J.S., 1987a. Entropy and information: suggestions for common language. Phil. Sci. 54, 176–193.

Wicken, J.S., 1987b. Evolution, Thermodynamics, and Information: Extending the Darwinian Program. Oxford University Press, New York.

Wiener, N., 1948. Cybernetics: or Control and Communication in the Animal and the Machine, 2nd edn. MIT Press, Cambridge, MA.

van Workum, M., van Dooren, S.J.M., Oldenburg, N., Molenaar, D., Jensen, P.R., Snoep, J.L., Westerhoff, H.V., 1996. DNA supercoiling depends on the phosphorylation potential in *Escherichia coli*. Mol. Microbiol. 20 (2), 351–360.

Yockey, H.P., 1992. Information Theory and Molecular Biology. Cambridge University Press, Cambridge.

Zgierski, M.Z., 1975. Mechanisms of conformon creation in α-helical structures. J. Theor. Biol. 55, 95–106.

Zurek, W.H., 1990. Complexity, Entropy and the Physics of Information. Addison-Wesley, Reading, MA.